CEST Imaging Reveals Dynamic Changes of Implanted Hydrogel Scaffold in vivo

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INTRODUCTION

As our knowledge of stem cell biology is increasing at an unprecedented pace, stem cell transplantation for therapeutic purposes has become a promising approach to treat a variety of neurodegenerative diseases. One critical issue in cell transplantation is the substantial loss of transplanted cells in host tissues due to apoptosis or necrosis (1, 2). Among the many ways to address this issue, hydrogel scaffolds have been demonstrated to be valuable in improving survival of transplanted cells by shielding the cells from a hostile microenvironment or promoting gas and nutrients exchange between graft and host (3). However, the microenvironment created by hydrogel scaffolds is amenable to changes due to interactions with encapsulated stem cells or surrounding host tissue, which may cause unwanted side effects on grafted cells. To address this problem, we propose to use CEST MRI, a powerful tool for the acquisition of molecular information, to monitor dynamic changes in hydrogel scaffolds in vivo.

MATERIALS AND METHODS

Hydrogel preparation and implantation: Injectable hyaluronic acid (HA)-based hydrogel Hysterm-C hydrogel (Glycosan Biosystem, Inc.) consists of HA, gelatin and crosslinker (PEGDA). Gel components were combined immediately prior to transplantation, which still allowed for 5-10 min of mixing cells before injection without gel solidification. Freshly prepared hydrogel alone or hydrogel encapsulated, luciferase expressing C17.2 mouse neural stem cells were stereotactically injected (3 μl gel alone or gel containing 1.5x10^7 cells) into the striatum of immune-deficient rag2-/- mice (n=3). A second group of mice (n=3) were injected with the same amount of non-encapsulated cells. Optical imaging and histology: Bioluminescence imaging (BLI) was performed using a Xenogen IVIS2000 system after i.p. injection of luciferin (150 mg/kg). At days 1 and 7, seven mice were sacrificed by transcardial perfusion with paraformaldehyde followed by post-fixation of brain tissue, dehydration and cryosectioning at 25 μm. Tissue sections were immunostained for β-galactosidase, a reporter gene allowing for detection of C17.2 cells. MRI: In vivo MRI experiments were performed under isoflurane anesthesia on a 9.4T Bruker BioSpec (Bruker) scanner at 1 hour and 7 days post-scaffold implantation. A modified RARE sequence (RARE factor 4, TR/TE=5000/16ms) including a magnetization transfer (MT) module (B1 = 3 μT, 3sec) was used to acquire CEST-weighted images from -4ppm to 4ppm (step=0.3 ppm) around the water resonance (0ppm). In vivo CEST MRI experiments were performed on an 11.7T Bruker Avance spectrometer with the following parameters: RARE (16), TR/TE=6000/9.3ms, B1=3.0μT/3sec (-5ppm to -5ppm, 0.2ppm steps). Data processing was performed using custom-written scripts in Matlab.

RESULTS AND DISCUSSION

BLI indicated that transplanted C17.2 cells survived well in both hydrogel-encapsulated implants and implants without scaffold (Fig.1A), indicating that the hydrogel scaffold does not have a detrimental effect. Histology demonstrated that transplanted cells initially distributed homogenously throughout the scaffold, and then (at day 7) migrated extensively out of the scaffold into the brain parenchyma (Fig.1B). Such immediate and extensive migration was unexpected because it is believed that gel-encapsulated cells remained entrapped and locally clustered for extended time periods. We then performed a phantom CEST MRI study on the molecular components of the scaffold (i.e., HA, gelatin, and PEGDA). Interestingly, the highest CEST-MRI contrast was detected from gelatin showing high MTR asymmetry values at 1.8 and 3.6 ppm (Fig.2). CEST MRI was then performed on scaffold implanted mice at 1 hour (day 0) and day 7 after transplantation of scaffold. Consistent with the phantom study, CEST signals at 1.8 and 3.6 ppm were easily identified in brains at day 0 (Fig.3, 1st row). Surprisingly, a dramatic drop in CEST-MRI contrast was obtained at day 7 (Fig.3, 2nd row), though the physical structure of the scaffold was maintained as detected histologically (Fig.3, 2nd row, inset). According to the phantom study, CEST contrast was primarily derived from gelatin, and therefore the decay in those signals suggests degradation of gelatin in this scaffold in vivo. Histological detection of gelatin would validate this hypothesis, but the results are consistent with the histological cell migration at day 7. Given that gelatin provides anchoring sites for cells within the scaffold, the degradation of gelatin could hinder the ability of the scaffold to keep cells immobile, which could potentially lead to migration of encapsulated cells out of the scaffold. The ubiquitous distribution of matrix metalloproteinases with collagenase activity in living tissues including the brain (4) may be responsible for degradation of gelatin in vivo.

CONCLUSION

The use of biomaterials for stem cell encapsulation and improvement of cell survival is on the rise. By providing molecular information on the composition and degradation of scaffold materials, CEST MRI could become a valuable tool for studying dynamic changes in scaffolds in vivo and allow further optimization of implantation strategies aimed at improved stem cell therapy.

Reference

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Fig 1: A: BLI of implanted cells (blue: cells only; pink: encapsulated cells); B: Histology shows cell migration towards the scaffold periphery at day 7 post-injection

Fig 2. CEST MRI phantom study of molecular components of the scaffold. Gelatin gives a strong signal at 1.8 ppm and 3.6 ppm.

Fig 3. In vivo CEST MRI of scaffold over time. On day 7, there was a dramatic drop in CEST signal at 1.8 ppm and 3.6 ppm, though the hydrogel was still present as revealed by histological staining (inset).